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Cytogenetic Studies on Wild *Chrysanthemum* sensu lato in China. II. A Natural Hybrid between *Dendranthema indicum* (2n = 36) and *D. vestitum* (2n = 54) from Hubei Province

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中国産野生キク属（広義）の細胞遺伝学的研究. II. 湖北省で採集されたシマカンギク（2 n=36）と ウラゲノギク（2 n=54）との自然雑種

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A natural hybrid between *Dendranthema vestitum* and *D. indicum* was newly recorded in Hubei Province, China. It had various intermediate characters between the two parental species such as leaf shape pinnatilobed to pinnatiparted with truncate to obtuse base, morphology and density of T-shaped trichomes on leaves, size of pollen grains. However, it had white-colored ligules similar to those of *D. vestitum*. This plant also showed intermediate features of the chromosome number of $2n=45$ and the karyotype of hybrid features between *D. vestitum* ($2n=54$) and *D. indicum* ($2n=36$). Moreover, the plant's meiosis in pollen mother cells exhibited configurations of 12 to 18 bivalents with some multivalents and univalents in combination at diakinesis, lagging chromosomes at anaphase I, and chromosome bridges and micronuclei at telophase I. Its pollen stainability was relatively high (87.6%).

(Continued from J. Jpn. Bot. **66**: 199–204, 1991)

Natural and/or artificial hybrids of more than 50 combinations in the Japanese *Dendranthema* have been recorded (Nakata et al. 1987). In contrast, only two natural hybrids and one artificial hybrid in the Chinese *Dendranthema* have been recorded [Shih and Fu 1983, Chen et al. 1964 (cited in Li et al. 1983)]; the natural hybrids of *D. indicum* (L.) Des Moul. × *D. lavandulifolium* (Fisch. ex Trautv.) Ling et Shih and *D. chanetii* (Levl.) Shih × *D. naktongense* (Nakai) Tzvel., and the artificial hybrid of *D. pekinense* [*D. indicum* var. *acutilobum* (= *D. indicum*) × *D. erubescens* (= *D. chanetii*)].

During the course of the second field study in China in 1989 for the Monbusho International Scientific Research Program – Joint Research, a natural hybrid between *Dendranthema indicum* and *D. vestitum* (Hemsl.) Ling was newly collected in the suburbs of Yichang City, Hubei Province, China, by M. Nakata. Z.J. Qiu and H. Uchiyama. This paper deals with morphology and cytology in this natural hybrid that clarify and justify its true-hybridity. Taxonomic description of this hybrid will be published elsewhere.

Materials and methods

The natural hybrid between *Dendranthema indicum* and *D. vestitum* (Fig. 1) was discovered within a *D. vestitum* population in Jiangjiawan, about 10 km northeast of Yichang City, Hubei Province (cf. Nakata et al. 1991), where some *D. indicum* grew in the neighborhood. Two ramets (Accession nos.: Nakata 9274 and 9275) were obtained from this plant. Additionally, ten individual plants of *D. indicum* and 18 individual plants of *D. vestitum* were collected in the same locality to detect hybridization and perhaps introgressive hybridization.

For SEM observation on leaf trichomes, winter-rosette leaf was obtained from each cultivated plant of the natural hybrid and its putative parents, *Dendranthema indicum* of Nakata 9289 and *D. vestitum* of Nakata 9265. Those leaves were fixed with FAA [formalin : acetic acid : 70% ethyl alcohol = 5: 5: 90 (v/v/v)], dehydrated through an ethanol series and treated with the critical point drying method. After sputter coated with gold, they were examined with a scanning electron microscope (JEOL JSM-25SIII).

Chromosome preparations, measurements and nomenclature in mitosis (meristematic cells in root tips) and meiosis (pollen mother cells = PMC's) in the three taxa were same as those described in Nakata et al. (1991).

Mature pollen grains were harvested from fully bloomed flower-heads of the natural hybrid-plant, *D. indicum* (Nakata 9276) and *D. vestitum* (Nakata 9263) fixed in 70% ethanol, and stained with Cotton Blue in lactophenol. Over 2,000 pollen grains were counted for stainability. Diameters of pollen grains (the external shape was almost non-angular and globular with numerous crests and spines) were measured on the basis of the photographs by using a IBAS-2 image analyzing system.

The voucher specimens were deposited in the Herbarium, Institute of Botany, Academia Sinica (PE), Beijing.

Observations and discussion

1) Morphology. Among the intermediate characters of the natural hybrid between *Dendranthema indicum* and *D. vestitum*, the most distinct character was leaf shape. *Dendranthema indicum* had pinnatifid or pinnatiparted (5-lobed) leaves with cordate to truncate base and well noticeable petioles. On the other hand, *D. vestitum* had elliptic, oblong-ovate, ovate or rhombate leaves with entire, crenate or slightly dentate margin and short petioles which sometimes could not be distinguished from acute or attenuate leaf base. The natural hybrid had pinnatilobed or pinnatiparted leaves with truncate to obtuse base

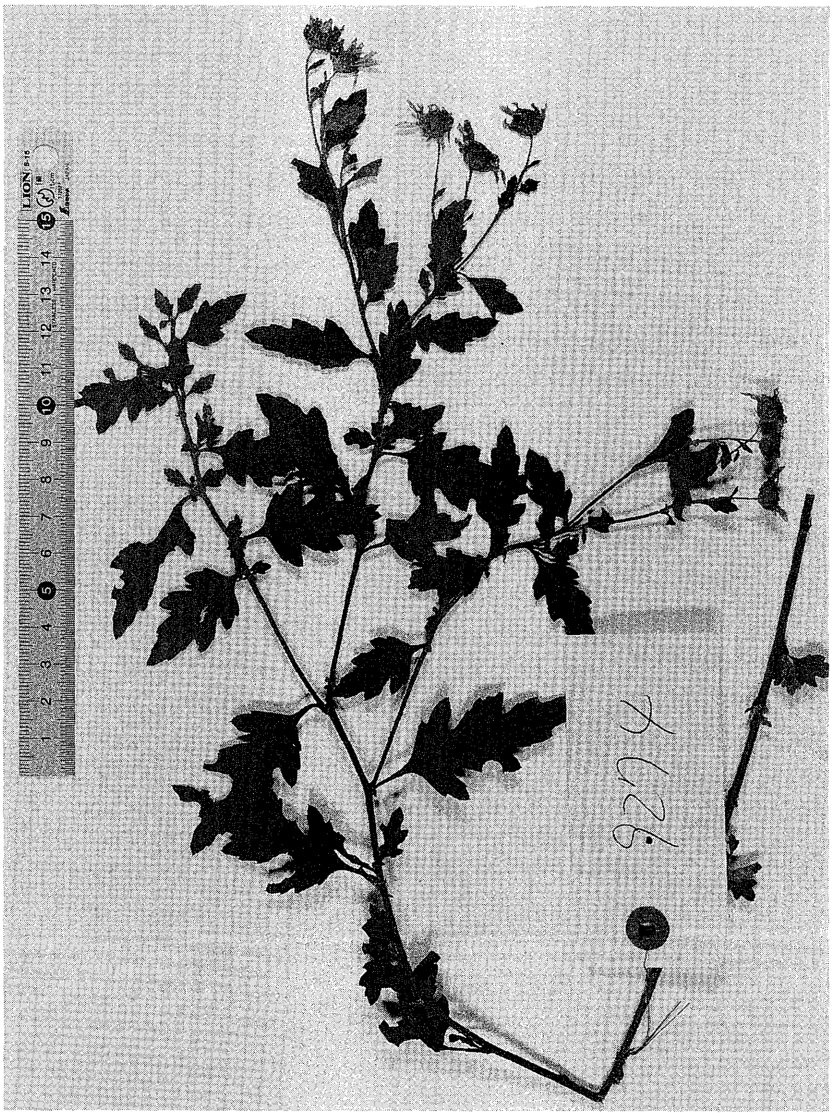

Fig. 1. The newly discovered natural hybrid (Nakata 9274) between *Dendranthema indicum* and *D. vestitum*.

and petioles of ca. 1cm long (Fig. 1). The flower head of the natural hybrid (Fig. 2. C, F) was similar in size to that of *D. indicum* (Fig. 2. A, D), but was identical in white-colored ligulate corolla with *D. vestitum* (Fig. 2. B, E).

Figure 3 shows trichomes on the leaf surface of *Dendranthema indicum* (A), *D. vestitum* (B) and their natural hybrid (C). Sizes and densities of T-shaped multicellular trichomes among the three taxa were different; the lengths of the uppermost lateral cells in *D. indicum* were short (200–300 μm), those in *D. vestitum* long (500–600 μm) and those in the natural hybrid intermediate (300–400 μm); the density of trichomes in *D. vestitum* was apparently higher than that of *D. indicum*, and the natural hybrid was intermediate between them.

2) Mitotic chromosomes. The chromosome number of the natural hybrid was $2n=45$ (Fig. 4),

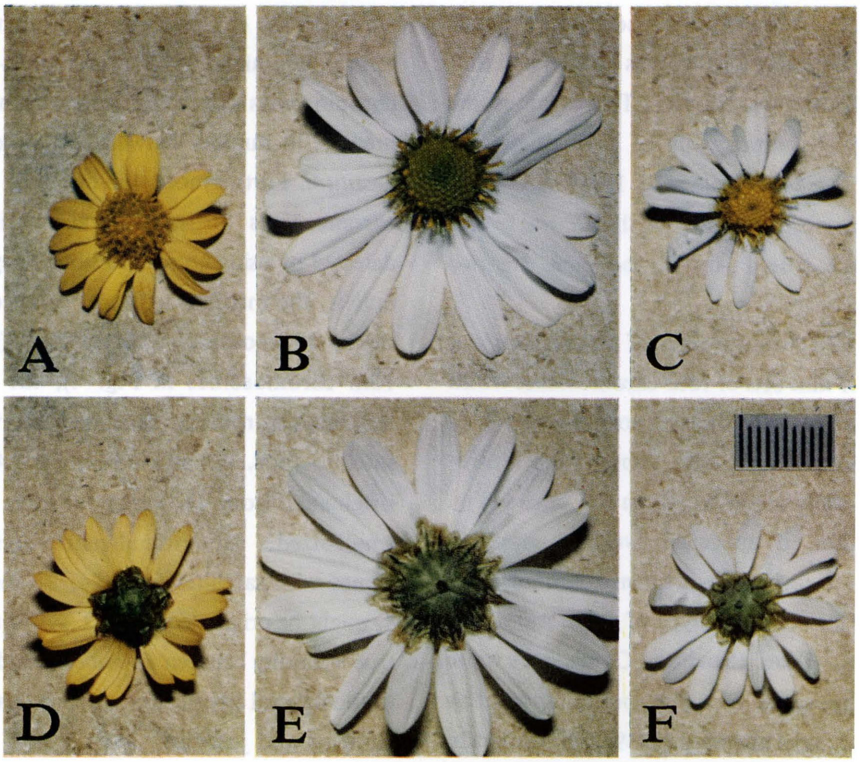

Fig. 2. Flower heads of *Dendranthema indicum* (Nakata 9276, A and D), *D. vestitum* (Nakata 9302, B and E), and the natural hybrid (Nakata 9275, C and F); upper (A–C) and lower (D–F) views. The photographs were taken at Yichang City on November 16, 1989. Scale = 1 mm.

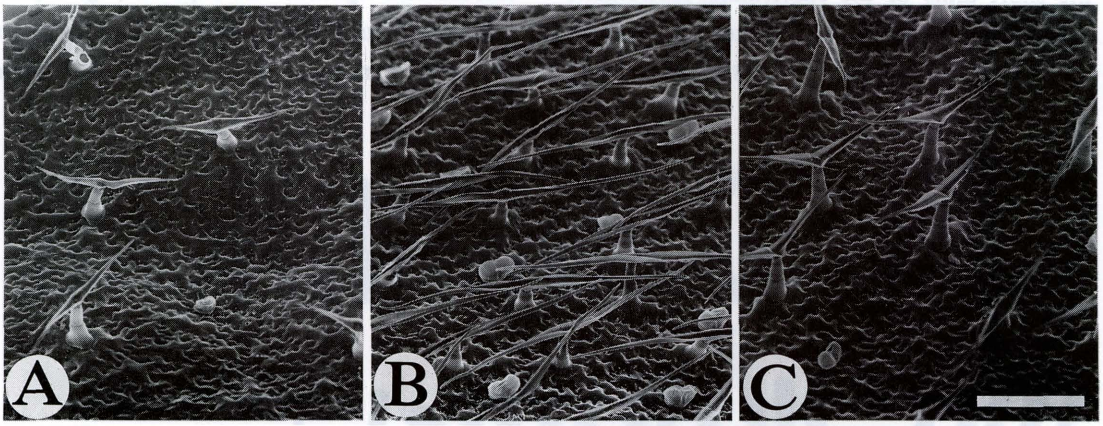

Fig. 3. Trichomes on the leaf surface of *Dendranthema indicum* (A), *D. vestitum* (B) and the natural hybrid (C). Scale bar = 200 μm.

while that of *D. indicum* in seven individuals collected in the same locality was 2n = 36 and that in one individual was 2n = 37. The chromosome number of *D. vestitum* in 12 individuals was commonly 2n = 54 (Nakata et al. 1991). According to those chromosome numbers, the natural hybrid could be the true F_1-hybrid between the two species.

The 2n = 36 chromosomes of *D. indicum* was composed of nine sets of four chromosomes which were classed by centromeric position; the four smallest chromosomes were subterminal centromeric and easily identified from the others (Fig. 5A). Satellites, if appeared, were observed in submedian- to median-centromeric chromosomes (Fig. 5A). Thus, the karyotype of *D. indicum* from Jiangjiawan, Hubei Province, China, was quite similar to that of the Japanese species reported by Shimotomai and Takemoto (1939) and Taniguchi (1987). According to Nakata et al. (1991), the karyotype of *D. vestitum* (2n = 54) was characterized by six medium-sized, subterminal-centromeric chromosomes (Fig. 5B). Satellites were located on the short arms of the subterminal-centromeric chromosomes and/or small-sized submedian-centromeric chromosomes (Fig. 5B). The 2n = 45 chromosome complement of the natural hybrid clearly contained five subterminal-centromeric chromosomes (Fig. 5C), that means the intermediate in number between *D. indicum* and *D. vestitum*. Furthermore, the origin of some of the chromosomes of the natural hybrid could be detected by using above mentioned key characters; that is, the medium to large, submedian-centromeric chromosomes with satellites (the 8th and 10th chromosomes in Fig. 4), the small, subterminal-centromeric chromosomes without any satellite (nos. 40 and 43 in Fig. 4) might be originated from *D. indicum*; while medium to small-sized, subterminal-centromeric chromosomes with satellites (nos. 24, 27 and 42 in Fig. 4) might be originated from *D. vestitum*. Thus, the true-hybridity between *D. indicum* and *D. vestitum* in the natural hybrid seemed to be karyotypically clarified and justified.

3) Microsporogenesis and pollen grains. Meiotic behavior was studied in PMC's in the natural hybrid. At pachytene stage, the majority of the chromosome segments was paired, while some chromosome segments were unpaired (Fig. 6A). At diakinesis (Figs. 6B, C), although 12–18 bivalents were seen, some multivalents and univalents were

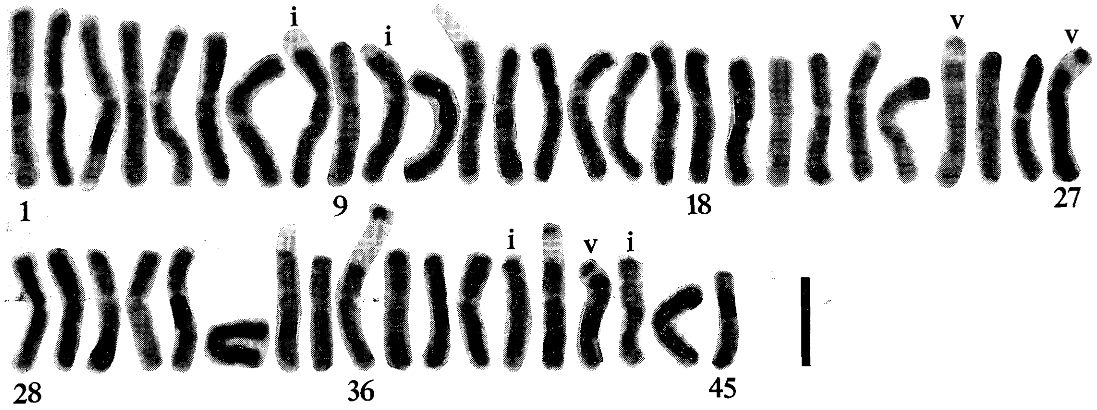

Fig. 4. Individual chromosomes of the natural hybrid between *D. indicum* and *D. vestitum* arranged by the decreasing order of the length. The marks "i" and "v" indicate the speculated origin of the chromosomes of *D. indicum* and *D. vestitum*, respectively. The scale bar = 5 μm.

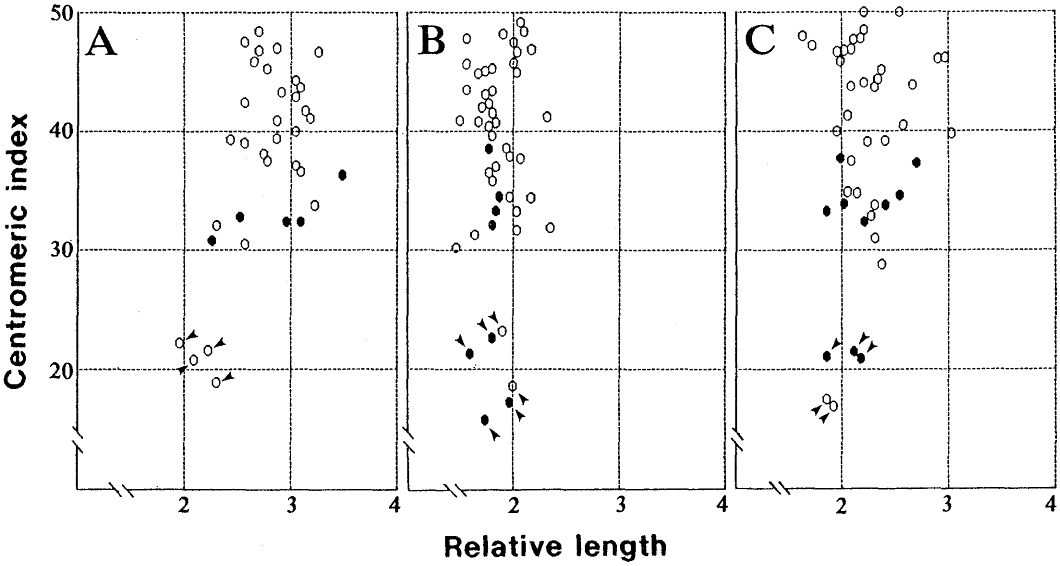

Fig. 5. Scatter diagrams showing chromosome composition of *D. indicum* (A), *D. vestitum* (B) and the natural hybrid (C). The diagrams were drawn based on the measurements on good metaphase plates respectively. Arrowheads indicate subterminal-centromeric chromosomes. Solid circles are the chromosomes that have satellites.

observed. Precise chromosome configurations could not be generally analyzed due to stickiness of the chromosomes, excepting three PMC's displayed quadrivalents (1–2), trivalents (1–4), bivalents (12–18) and univalents (1–2). Among the 20 PMC's at metaphase I observed, only three were normal and the others were abnormal showing one to seven unpaired chromosomes (Fig. 6D). The majority of the PMC's at anaphase I to telophase I studied showed two to nine lagging chromosomes (Fig. 6E). Moreover, the majority of the PMC's at anaphase II studied showed lagging chromosomes or chromosome bridges (Fig. 6F).

At tetrad or 4-celled stage, 43 out of the 126 PMC's (34.1%) observed had four cells with the normal nucleus. In contrast, the other 83 PMC's (65.9%) showed abnormal features having one to four micronuclei (57.9%) (Fig. 6G) or forming triads (7.9%) (Fig. 6H). Thus, although the PMC's of the natural hybrid showed highly abnormality, the pollen grains (Fig. 5I) showed relatively high stainability (87.6%) compared with those of *Dendranthema indicum* (94.1%) and of *D. vestitum* (97.2%).

The means ± standard deviations and the ranges (in parenthesis) of pollen grain diameter in *Dendranthema indicum, D. vestitum* and the natural hybrid were 30.5 ± 1.7 μm (26.8–36.0 μm), 35.7 ± 1.6 μm (32.0–42.9 μm) and 32.0 ± 8.0 μm (20.9–39.2 μm), respectively. Thus, sizes of pollen grains of the natural hybrid varied widely, but however, the mean value was the intermediate between that of its parental species. The unequal chromosome distribution in PMC's of the natural hybrid described above could be reflected to the wide range of pollen size.

Artificial hybrid between the tetraploid (2n = 36) *Chrysanthemum indicum* L. (= *D. indicum*) and the hexaploid (2n = 54) *C. japonense* Nakai [= *D. occidentali-japonense* (Nakai) Kitam.] made by Shimotomai (1933) is referable to the present natural hybrid. *Dendranthema indicum* has yellow ligulate corolla, while *D. occidentali-japonense* has

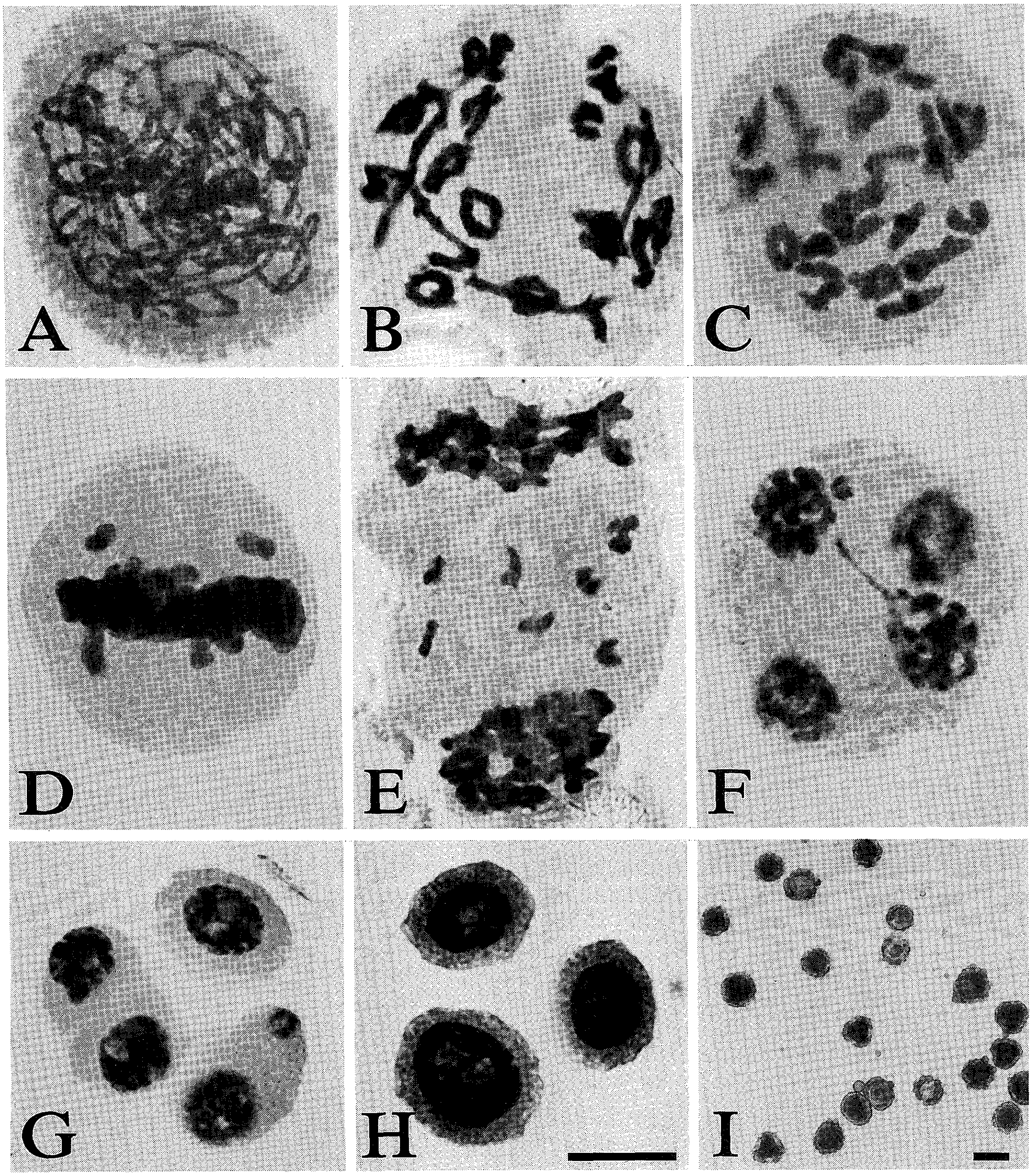

Fig. 6. Microsporogenesis in the natural hybrid between *Dendranthema indicum* and *D. vestitum*. A: Early pachytene showing paired (thick) and unpaired (thin) chromosome segments. B and C: Diakinesis showing 12 (B) and 18 (C) bivalents. D: Metaphase I. E: Anaphase I showing eight laggards. F: Telophase II showing a laggard and a bridge. G: Tetrad with micronucleus. H: Triad. I: Pollen grains stained with Cotton Blue in lactophenol. The scale bar in H = 10 μm for A–H. The scale bar in I = 10 μm.

white ligulate corolla like *D. vestitum*. The artificial F_1-hybrids between *D. indicum* and *D. occidentali-japonense* made by Shimotomai (1933) had the chromosome number of 2n = 45 and the white ligulate corolla. *Dendranthema* could have the same hereditary mechanism which could be adapted to the present phenomenon. Thus, the natural hybrid studied here must be the true

F_1-hybrid between *D. indicum* and *D. vestitum*.

The F_1-hybrid between *D. indicum* and *D. occidentali-japonense* of Shimotomai (1933) usually had one or two trivalent(s), eight univalents and seven bivalents. However, the present observation on the multivalent formation in the natural hybrid was different from that of Shimotomai (1933). Further observations on meiosis in the natural hybrid are necessary to clarify this problem.

In Hebei, Shanxi and Liaoning Provinces, China, *Dendranthema chanetii* (2n = 36, 54) and *D. lavandulifolium* (2n = 18) were sympatric to each other, and rarely mixed with *D. indicum* (2n = 36). Since any hybrid among them have not been found, there may be some isolation mechanisms among those species. On the contrary, *D. vestitum* and *D. indicum* seemed to grow in a little different habitat in Yichang City from each other; the former was seen on foothills while the latter was mostly observed on loadsides. In general, population of *Dendranthema* could easily expand by human disturbances such as deforestation and cutting a path. The locality where the natural hybrid was found might be an overlapping edge of the populations of *D. vestitum* and *D. indicum*, either or both of which have expanded. Additional records on the natural hybrid between the two species may be expected.

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要　旨

中国湖北省宜昌市郊外の，シマカンギク *Dendranthema indicum* L. 個体群が隣接するウラゲノギク *D. vestitum*（Hemsl.）Ling個体群中で，両種の自然雑種が1個体採集された．自然雑種は小型の白色舌状花と中－浅（5）裂する葉を持ち，葉の表面のT字状の毛状突起の形態と密度，花粉粒の大きさなどで両種の中間的形態を示した．自然雑種の染色体数は2n=45であったが，これは同時に採集されたシマカンギク，ウラゲノギクの染色体数がそれぞれ2n=36（6個体．1個体は2n=37），2n=54（12個体）であったので，両種の雑種第1代における予想染色体数と一致する．シマカンギク，ウラゲノギク，自然雑種の染色体組中には次端部動原体型染色体がそれぞれ4個，6個，5個観察され，核型からも両種の雑種であることが確かめられた．花粉母細胞における

減数分裂および花粉形成では，移動期において二価染色体（12－18個）の外に多価，一価が形成され，その後，遅滞染色体，染色体橋，小核形成などの異常が観察されたが，花粉染色性は87.6%と比較的高い値を示した.